Conventional and extended intramammary therapy of persistent subclinical mastitis using nafcillin-penicillin-dihydrostreptomycin in lactating dairy cattle

Kasravi, R.[1*]; Bolourchi, M.[1]; Farzaneh, N.[2]; Seifi, H. A.[2]; Barin, A.[1]; Hovareshti, P.[1] and Gharagozlou, F.[1]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

[*]**Correspondence:** R. Kasravi, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. E-mail: rkasravi@hotmail.com

(Received 7 Feb 2010; revised version 8 Jan 2011; accepted 16 Jan 2011)

Summary

The objective of the present study was to compare the efficacy of conventional and extended intramammary (IMM) therapy of persistent subclinical mastitis in lactating dairy cattle using nafcillin-penicillin-dihydrostreptomycin combination (NPD). Sixty-five dairy cows with 126 infected quarters were enrolled in the study. Infected cows were allocated randomly to 1 of 3 different treatment regimens: (1) conventional group: NPD administered IMM 3 times at 24-h intervals (20 infected cows, 43 intramammary infections [IMI]), (2) extended group: NPD administered IMM 6 times at 24-h intervals (23 cows, 43 IMI), and (3) untreated control group (22 cows, 40 IMI). The overall bacteriological cure (BC) rates for subclinical IMI were 86.04%, 100%, and 20% for the conventional, extended and the control groups, respectively; indicating a higher BC rate ($P<0.0001$) for the treated groups than the control group. Significant difference ($P=0.029$) concerning the BC rate was also observed between the extended and the conventional groups. Significant difference ($P=0.0021$) in somatic cell count (SCC) was detected between the extended and the control group. Fat percentage increased in the conventional ($P=0.029$) and in the extended ($P<0.0001$) groups, and protein percentage increased only in the extended group ($P=0.0016$). There was no significant difference in posttreatment milk production between the groups ($P>0.05$). Results of this study indicate that NPD therapy was effective in eliminating subclinical IMI in lactating dairy cows, and that extended therapy enhanced BC rate and reduced SCC.

Key words: Dairy cow, Subclinical mastitis, Extended therapy

Introduction

Mastitis – inflammation of the mammary gland – is caused by several species of bacteria, fungi, mycoplasmas and algae. Subclinical mastitis are those for which no visible changes occur in the appearance of milk or the udder, but milk production decreases, somatic cell count (SCC) increases, pathogens are usually present in the secretion, and the milk composition is altered (Batavani *et al*., 2007). Many intra-mammary infections (IMI) prove to be persistent for a considerable length of time resulting in high SCC and decreased milk yield. Any IMI may lead to development of clinical mastitis (CM) and spread of mastitis pathogens from infected to uninfected mammary quarters within the herd (Oliver *et al*., 2004b; Swinkels *et al*., 2005a, b). Antibacterial therapy is an important part of a mastitis control program in dairy cattle (Kasravi *et al*., 2010). Treatment of many subclinical IMIs is often postponed until the dry period (Hillerton and Berry, 2003) or until a clinical flare-up is observed. However, with the increasing demand to produce milk with a low bulk tank somatic cell count (BTSCC), it may not be economically justifiable to wait until dry-off (Deluyker *et al*., 2005). Thus, there is an interest in adopting effective treatment

strategies. Treatment may be economically justifiable if the benefits of treatment of subclinical mastitis outweigh the costs (Hillerton and Berry, 2003), or when clinical cases (St. Rose *et al*., 2003) or spread of infection (Zadoks *et al*., 2002) can be prevented. Recent studies have shown that treatment of subclinical IMI caused by environmental streptococci may contribute to the prevention of CM and streptococcal transmission (Zadoks *et al*., 2001, 2003). The combination of nafcillin with penicillin and dihydrostreptomycin broadens the antimicrobial spectrum of the drug (Phillips, 1979). Although NPD has been used as an IMM preparation for more than 3 decades in many countries, to the best of our knowledge, this is the first study which compares the efficacy of conventional and extended IMM therapy of subclinical IMI in lactating dairy cattle using NPD. The objectives of the present study were to compare the efficacy of nafcillin-penicillin-dihydrostreptomycin (NPD) intramammary (IMM) therapy for treatment of naturally occurring, persistent subclinical mastitis in lactating dairy cattle caused by a variety of mastitis-causing pathogens during various stages of lactation.

Materials and Methods

Experimental location and animals

The study was conducted in a closed, commercial, large Holstein dairy with an average of 1100 lactating dairy cows in summer 2007. All lactating cows were milked three daily. All lactating cows with a composite milk SCC≥150,000 cell/mL (based on Animal Breeding Center of Iran [ABC] monthly test data) were considered for inclusion.

Enrollment, sample collection and evaluations

Mammary quarter foremilk samples were obtained for microbiological and SCC analyses on 2 occasions 7 days apart. Sixty five dairy cattle with 126 infected quarters were enrolled in the study based on composite milk SCC≥150,000 cell/mL at the last test-day record, positive Californian mastitis test (CMT) results [scores T, 1, 2, or 3 as described by Schalm *et al*. (1971)] with a commercial reagent (Delaval mastitis test, DeLaval, Wroclaw, Poland) at the time of first pretreatment sampling, quarter milk SCC≥200,000 cell/mL and isolation of the same mastitis pathogen in the 2 samples obtained 7 days apart. Sampling and microbiological procedures were conducted in accordance with National Mastitis Council (NMC) guidelines (Oliver *et al*., 2004c). A definitive identification of the suspected bacterium was made using biochemical tests specific for that organism as described by Quinn *et al*. (1994, 2002). Quarter milk SCC was measured using the fluoro-opto-electronic cell counting method (COMBIFOSS 5000, Fossomatic, Foss Electric, Denmark). Quarters yielding more than one pathogen, different or no pathogens at any stage of pretreatment samplings as well as quarters with teat lesions (with the exception of teat-end hyperkeratosis) were excluded from the study. Cows were also excluded from the study if they were systemically ill, had clinical mastitis, had received antibiotics or antiinflammatory drugs in the previous 30 days, had reached a low milk yield (≤10 kg/day), had DIM of less than 7 days, or were to be dried off within the next 60 days. Mammary quarter foremilk samples were collected 14 and 28 days after the last treatment for microbiological and SCC evaluations. Bacteriological cure (BC) was defined as a treated infected quarter that was bacteriologically negative for the presence of previously isolated bacteria at 14 and 28 days after the last treatments. Cytological cures (CC) were defined as quarter SCC reached below 100,000/mL at day 28 after the last treatment. Combined cures (bacteriological + cytological; BCC) were defined as a treated infected quarter that was bacteriologically negative for the presence of previously isolated bacteria at 14 and 28 days after the last treatments; also, SCC reached below 100,000 cell/mL at 28 days after the last treatment. New infections (NI) were defined as infections that identified - at, and persisted through both posttreatment samplings - with the same pathogen, but were caused by different bacteria from the previously (pretreatment) isolated ones. Composite milk yield (as kg/cow per test day), and fat and protein

percentages (as percent/cow per test day) were compared between the treatment groups using the herd test-day data at the last pretreatment and the first posttreatment ABC records.

Treatments

Infected cows were allocated randomly to 1 of 3 treatment groups: (1) Conventional treatment group: a commercial IMM preparation consisting of 100 mg of sodium nafcillin plus 180 mg of sodium penicillin, and 100 mg of dihydrostreptomycin sulphate (Nafpenzal MC; Intervet International, Boxmeer, Holland) per 3-ml plastet administered IMM 3 times at 24-h intervals (20 infected cows, 43 IMI; mean DIM of 277 with a range of 112 to 609-d; median parity of 2 with a range of 1 to 6), (2) Extended regimen group: 100 mg of sodium nafcillin, 180 mg of sodium penicillin, and 100 mg of dihydrostreptomycin sulphate administered intramammarily 6 times at 24-h intervals (23 infected cows, 43 IMI; mean DIM of 284 with a range of 124 to 623-d; median parity of 2 with a range of 1 to 8). The manufacturer recommends infusion of one syringe of the product into each affected mammary quarter at 24-h intervals. (3) Control group consisted of 22 cows with 40 IMI (mean DIM of 224 with a range of 19 to 474-d; median parity of 2.5 with a range of 1 to 7) which were considered as an untreated negative control group.

Statistical analysis

Because milk SCC, yield, fat, and protein were measured over time, a repeated measures approach using ANOVA with Mixed linear models in SAS (2001) was used (fixed effects of treatment and covariates, random effects of cow and quarter). Because there were three samples for each cow for SCC determination, a Bonferroni correction of the probability value was used ($P<0.016 = 0.05$ divided by 3). The treatment effect on the proportion of quarters with BC, CC, BCC, and NI were evaluated with Mantel-Haenszel Chi-square statistics with PROC FREQ statement of SAS (2001). For these comparisons, $P<0.05$ was considered to be significant.

Results

Bacteriological cure rates

Most IMI were due to CNS (58.73%: 74/126), coliforms (13.49%: 17/126), and environmental streptococci (11.11%: 14/126). The distribution of pathogens causing subclinical IMI across the treatment groups is presented in Table 1. The overall BC rate for all IMI was 86.04%, 100%, and 20.00% for the conventional, extended, and the control groups, respectively. Differences in BC rate were detected between both treatment regimens and the control group ($P<0.0001$), and between the extended and the conventional treatment groups ($P=0.029$) (Table 2). Results of the BC rate for different bacterial groups are presented in Table 3. Both treatment groups had higher BC rates than the control for CNS. However, the BC rate for the extended treatment group

Table 1: Distribution of pathogens causing subclinical intramammary infections across the treatment groups

Pathogen*	Treatment groups			
	Conventional	Extended	Control	Total
CNS[1]	21	29	24	74
C. bovis[2]	5	1	5	11
Environmental streptococci[3]	8	2	4	14
Coliforms[4]	6	7	4	17
S. aureus[5]	2	2	2	6
Others	1	2	1	4
Total	43	43	40	126

[1]CNS: Coagulase-negative staphylococci (*Staph. hyicus*, *Staph. auricularis*, *Staph. kloosi*, *Staph. hominis*, *Staph. muscae*, *Staph. carmosus*, *Staph. saprophyticus*, *Staph. epidermidis*, *Staph. milleri*, *Staph. caseolyticus*, and *Staph. sciuri*). [2]*C. bovis*: *Corynebacterium bovis*. [3]*Streptococcus dysgalactiae* (predominant spp.) and *Streptococcus equinus*. [4]*E. coli* (predominant spp.), *Enterobacter aerogenes*, and *Klebsiella pneumonia*. [5]*S. aureus*: *Staphylococcus aureus*. *The herd was free of *Streptococcus agalactiae* and *Mycoplasma bovis* IMI based on several individual and bulk tank milk cultures and serological tests

Table 2: Comparison of bacteriological cure (BC) rates for subclinical intramammary infections and somatic cell count (SCC) in treatment groups

NPD treatment group	BC rate[1]	SCC ($\times 10^3$)[2, 3]		
		Pretreatment	14 d posttreatment	28 d posttreatment
Conventional	86.04% (37/43)[†]	897.8 ± 1.3[a*]	226.8 ± 1.3[a**]	301.6 ± 1.3[ab]
Extended	100% (43/43)[‡]	425.8 ± 1.3[a]	224.7 ± 1.3[a**]	223.6 ± 1.3[b]
Control	20.00% (8/40)[§]	478.2 ± 1.3[a]	429.2 ± 1.3[a]	522.2 ± 1.3[a]

[1]Values with different symbols or letters at each column differ at two-sided P-value <0.0001. [2]SCC is presented as mean LSM ± SEM. [3]Although significant differences have been detected between 14- and 28-d posttreatment versus the pretreatment SCC values in both treated groups (P<0.0022), there were no differences between Day 14 and Day 28 SCCs in treatment groups. No differences were detected between the pre- and posttreatment SCC values in the control group. [*]Comparison between the conventional versus the extended and control groups for pretreatment SCC revealed a marginally nonsignificant difference at the level of P = 0.02. [**]Comparison between the extended and conventional versus the control groups for 14-d posttreatment SCC revealed a marginally nonsignificant difference at the level of P = 0.02

Table 3: Comparison of bacteriological cure rates of subclinical mastitis among treatment regimens for pathogen groups

NPD treatment group	Pathogens[1]					
	CNS[2] (74)	*C. bovis*[3] (11)	Environmental streptococci (14)	Coliforms (17)	*S. aureus*[4] (6)	Other pathogens (4)
Conventional	90.47%[a] (19/21)	100%[a] (5/5)	100%[a] (8/8)	66.66%[ab] (4/6)	0% (0/2)	100% (1/1)
Extended	100%[a] (29/29)	100%[ab] (1/1)	100%[ab*] (2/2)	100%[a] (7/7)	100% (2/2)	100% (2/2)
Control	25%[b] (6/24)	20.00%[b] (1/5)	0%[b] (0/4)	0%[b] (0/4)	0% (0/2)	100% (1/1)

[1]Different letters at each column significantly differ at two-sided P-value <0.05. [2]CNS: Coagulase-negative staphylococci. [3]*C. bovis*: *Corynebacterium bovis*. [4]*S. aureus: Staphylococcus aureus*. [*]Comparison between the extended and the control groups for environmental streptococci revealed marginally nonsignificant difference at the level of P = 0.06

was not higher than the conventional regimen. Comparison between extended and control groups for environmental streptococci revealed a marginally nonsignificant difference at the level of P=0.06.

Somatic cell count pattern

There were no significant differences in preenrollment SCC between the groups. Following treatment, SCC decreases in both treatment groups (P<0.01). There were no significant differences in SCC between the conventional and control groups at either posttreatment sampling (P>0.016). No significant difference was found between the extended and control groups at first posttreatment evaluation (P>0.016). However, a highly significant difference in SCC between the extended versus the control group was observed at the second posttreatment evaluation (P=0.0021). There were no significant differences in SCC between the two posttreatment samplings in any groups. The difference in SCC between the extended and conventional treatment groups was nonsignificant at both posttreatment evaluations (Table 2).

Cytological and combined cure rates

The CC in the conventional, extended and the control groups was 30.23% (13/43), 34.88% (15/43), and 2.50% (1/40), respectively. Highly significant differences were detected between the treated groups versus the control group (χ^2 = 8.63, P=0.0033). The difference between the extended and the conventional groups was nonsignificant. The BCC in the conventional, extended and the control groups was 30.23% (13/43), 34.88% (15/43), and 0.00% (0/40). Highly significant differences were detected between the treated groups versus the control group (χ^2 = 10.52, P<0.0012). The difference between the extended and conventional groups was nonsignificant.

New infection rates

The new infection rates in the conventional, extended and the control groups were 2.32% (1/43), 2.32% (1/43),

Table 4: Comparison of milk yield, fat, and protein percentages pre-, and posttreatment in treatment groups[1, 2]

NPD treatment groups	Pretreatment values[3]			Posttreatment values[4]		
	Milk	Fat	Protein	Milk	Fat	Protein
Conventional	27.19±1.71†	3.38±1.05^{a}	3.19±1.03^{a}	21.84±1.71‡	3.89±1.05^{b*}	3.35±1.03^{ab}
Extended	23.89±1.59†	3.12±1.05^{a}	3.15±1.03^{a}	20.57±1.59‡	4.09±1.05^{b**}	3.63±1.03^{b}
Control	27.48±1.66†	3.38±1.05^{a}	3.12±1.03^{a}	22.56±1.66‡	3.59±1.05^{a}	3.22±1.03^{a}

[1]There were no significant differences in pretreatment values between the groups. [2]Different letters or symbols at each row or column (for each variable) are significantly different at two-sided P<0.05. [3]Values at the last test day record before the beginning of the study. Milk yield is presented as LSM ± SEM (kg/test day per cow). Fat and protein are presented as LSM ± SEM (percentage/test day per cow). [4]Values at the first test day record after the end of the study period. [*]There was no significant difference in posttreatment fat values between the conventional and the control groups. [**]Comparison between the extended and the control groups for the posttreatment fat values revealed marginally nonsignificant difference at the level of P = 0.08

Table 5: The effects of predictor variables on the outcomes of the study (SCC, milk yield, milk fat percentage and milk protein percentage) at the entry level of the statistical model[1]

Predictor variable	P-values for outcome variables			
	SCC	Milk yield	Milk fat	Milk protein
Treatment group (tg)	0.03	0.03	0.26	0.59
Parity group (pg)[2]	0.06	0.34	0.18	0.90
DIM group (dg)[3]	0.32	0.0002	0.07	0.41
Bacterial group (bg)[4]	0.02	0.38	0.14	0.48
Day of sampling (d)[5]	<0.0001	<0.0001	<0.0001	0.004
tg * pg	0.63	0.19	0.98	0.98
tg * dg	0.54	0.73	0.21	0.50
tg * bg	0.01	0.49	0.25	0.81
tg * d	<0.0001	0.27	0.04	0.20

[1]All variables were offered to each model and then removed in a backward stepwise elimination approach. Interactions between treatment and the remaining significant variables were tested and included in the final model if significant. [2]Categorized as first and second, third or greater parity. [3]DIM at enrolment (≤100, 101 to 150, 151 to 200 and >200 DIM). [4]Grouped as CNS, *C. bovis*, environmental streptococci, coliforms, *S. aureus*, and others. [5]Pretreatment, 14- or 28-d posttreatment

and 2.50% (1/40), respectively. Significant differences were not observed between the groups.

Milk yield, protein, and fat percentages

There were no differences in preenrollment or posttreatment milk production between the groups (P>0.05). However, the posttreatment decrease in milk yield was significant for all groups (P<0.0001 for the conventional and control groups, and P=0.0006 for the extended group). There were no significant differences in preenrollment protein or fat percentages between the groups (P>0.05). However, fat percentage increased in the conventional (P=0.029) and in the extended (P<0.0001) groups, and protein percentage increased only in the extended group (P=0.0016) (Table 4).

The effects of predictor variables on the outcome variables of the study (SCC, milk yield, milk fat percentage and milk protein percentage) at the entry level of the statistical model are presented in Table 5.

Discussion

Results of the present study indicate that NPD therapy was effective in reducing milk SCC and eliminating subclinical IMI in lactating dairy cattle caused by different mastitis pathogens at various stages of lactation, and that extended therapy enhanced treatment efficacy. Previously, *in vitro* (Sampimon *et al*., 2007) and *in vivo* (Phillips, 1979; Ziv *et al*., 1981; Bolourchi *et al*., 1995; Shpigel *et al*., 2006) activity of the combination of nafcillin, penicillin, and dihydrostreptomycin have been shown against a variety of mastitis pathogens, especially staphylococci. The efficacy of conventional and extended IMM therapy

regimens against subclinical IMI has been previously demonstrated for ceftiofur and pirlimycin (Gillespie *et al*., 2002; Oliver *et al*., 2004b; Deluyker *et al*., 2005).

The overall BC rate for all subclinical IMI in this study was 86.04%, 100%, and 20% for the conventional, extended, and control groups, respectively. Significant differences in BC rate were detected between both treated groups versus the control group. In addition, the extended NPD treatment regimen significantly enhanced BC rate.

Results of previous studies on subclinical mastitis support the concept that extended therapy is more effective in eliminating subclinical IMI than standard treatment. This has been demonstrated for ceftiofur and pirlimycin against *Streptococcus uberis*, other environmental streptococci, and *S. aureus* (Owens *et al*., 1997; Gillespie *et al*., 2002; Oliver *et al*., 2004b; Deluyker *et al*., 2005).

In the studies conducted on experimentally-induced *S. uberis* clinical mastitis with pirlimycin or ceftiofur (Oliver *et al*., 2003; Oliver *et al*., 2004a), the enhanced BC rates were observed for extended treatment regimens. The SCC decreased significantly following therapy in quarters for which treatment was successful in eliminating *S. uberis*. However, there was no evidence suggesting that extended therapy with pirlimycin or ceftiofur resulted in a greater reduction in SCC than the conventional treatment regimens.

Both treatment groups had significantly higher BC rates than the control for CNS. However, the extended group did not have a significantly higher BC rate for the pathogen group than the conventional treatment group. On the contrary, low CNS bacteriological cure rate and its failure to increase with a longer duration of treatment, as well as significantly higher cure rates for *S. aureus* and environmental streptococci with extended therapy in comparison with standard therapy were found in a study on subclinical mastitis conducted by Deluyker *et al*. (2005).

Following the treatment SCC decreased significantly in both treatment groups. A highly significant difference in SCC between the extended and the control group was detected at the second posttreatment sampling. There were no significant differences in SCC between the two posttreatment samplings in the treatment groups. This is consistent with the result found on subclinical mastitis with pirlimycin (Deluyker *et al*., 2005). The differences in SCC between the extended and conventional treatment groups were nonsignificant at both posttreatment samplings in the present study. This is consistent with the results found in the previous studies on experimentally-induced *S. uberis* clinical mastitis (Oliver *et al*., 2003, 2004a), but in contrast with the result found on subclinical mastitis with pirlimycin (Deluyker *et al*., 2005). We found a lower than expected CC and BCC following the treatment in the conventional and extended treatment groups. This was caused by the fact that SCC did not reach the threshold of 100,000 cell/mL by the end of the study period in the majority of quarters in which IMI was successfully eliminated. Based on the NMC guideline, mammary quarters from which no microorganisms can be isolated and that have no history of recent infection will almost always have a SCC of less than 100,000 cell/mL (Harmon *et al*., 2001). It is stated that the range of time required for SCC to return to normal level may vary from a few days to a few months or even to the next lactation depending on the type of microorganism involved and the amount of tissue damage resulting from the infection (Bramley *et al*., 1996). Therefore, posttreatment samples may not be adequate or appropriately timed to capture desired or expected changes in SCC following BC.

Following the treatment, Fat percentage increased in both treated groups, and protein percentage increased only in the extended regimen group. These desirable changes can be attributed to the therapeutic effect of NPD on IMI. The test day milk production decreased significantly following the treatment in all groups including the control group in the present study. The most probable reasons seem to be the combined negative effects of heat stress and advanced lactation on milk production level in cows, especially in mid to late lactation (the majority of population in this study).

In the present study, the new infection

rates in the conventional, extended and the control groups were 2.32, 2.32, and 2.5%, respectively. Significant differences between the groups were not observed. In contrast with the observations of Gillespie *et al*. (2002), clinical mastitis (or deaths due to severe mastitis) was not observed in treated cows during the treatment or posttreatment period in our study. Although both studies were conducted during the summer months when dairy cattle were exposed to heat stress, the broad spectrum of NPD in comparison with a narrow spectrum of pirlimycin, could be the probable reason for this observation. Since we used naturally-occurring subclinical IMI as well as allocating the treatments before knowing the culture results, this study provided data on treatment efficacy on subclinical IMI when the causative agents are unknown.

It is worthy to mention that the effectiveness of conventional or extended therapy of subclinical mastitis must be weighed against a variety of factors. These factors include the costs associated with the therapy, loss of milk during the withholding period, risk of infecting the quarters by sequential intramammary infusions, increased milk production following the treatment, reduced spread of mastitis pathogens, reduced incidence of clinical mastitis, getting a bonus for low BTSCC, reduced culling of cows due to acute or recurrent mastitis, and public health concerns about using antibiotics in food animals. Further studies are needed to investigate these objectives.

The results of this study indicate that extended NPD therapy significantly enhanced treatment efficacy in comparison with conventional therapy. When the primary objective of the therapy is to raise milk production in the current lactation, antibiotic therapy of subclinical mastitis in mid to late lactating cattle as well as during heat stress is not recommended. Further studies are needed to elucidate the economic impacts of such treatments.

Acknowledgements

This investigation was funded by the Research Deputy of the University of Tehran (Project No.:7508036/6/3). Authors express their appreciation to Mr. Sh. Saffari (for kind provision of Nafpenzal MC), Mr. Salimi, Mr. Narimani, Dr. D. Nikjou, Mr. Sattari, Mr. Dehghan, Mrs. Sh. Noursalehi and Dr. Mehrnaz Rad for their kind support and assistance.

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